Effects of Some Materials Extracted from *Ajuga* Species on the Larvae of *Hyphantria cunea* and its Natural Enemies[1]

Chi Defu(迟德富)
Northeast Forestry University, Harbin 150040, P. R. China
Darvas Bela
Plant Protection Institute, Hungarian Academy of Sciences, Budapest, P.O.Box 102, h-1525, Hungary
Rafael Ocete Rubio
Applied Zoology Department, Biological Faculty, Sevilla University, Sevilla, Spain
Jin Chuanling
The Quarantine and Control Station, Liaoning Province, 110000, P. R. China

Abstract Six kinds of extracts drawn from *Ajuga multiflora* and *A. multiflora* var. *brevispicata and A. multiflora* var. *serotina* using methanol and acetone were used in this experiment to test their killing activity to the second instar larvae of *Hyphantria cunea* and their influence on its natural enemies. The average death rate caused by those extracts on the second instar larvae was 85.70%. The mortality rate caused by the extracts drawn with methanol was from 88.89% to 96.33%, which was significantly higher than that caused by acetone extracts. Those extracts were safe to *Trichogramma dendrolimi, Coccinella septempunctata,* the natural enemies of *H. cunea.* We did not found any evidence shown that those extracts had any influence the emergence and the development of *T. dendrolimi.* Those methanolic extracts gotten from *A. multiflora* and *A. multiflora* var. *brevispicata* had no significant effects on the mortality of the larvae and adults of *C. septempunctata.* Those extracts could be used in the control of *H. cunea* safely.

Key words: *Ajuga multiflora; Ajuga. multiflora* var. *brevispicata; A. multiflora var. serotina;Hyphantria cunea;* Extracts; Natural enemy; *Trichogramma dendrolimi; Coccinella septempunctata;* Control

Introduction

Ajuga species (Labiatae) common name bugle, which have been used to treat sore throats, inflammation, infectious disease, fevers, diabetes, hypertension and gastralgia in the Chinese folk medicine, also produce allelochemicals which affect postembryonic development and reproduction in invertebrate herbivores. Luteolin, apigenin and their glycosides; iridoides; catechin-like compound; caffeic, 4-caffeoylquinic and chlorogenic acids; and a significant quantity of diterpenoid neoclerodanes and phytoecdysteroids are formed as secondary metabolism of these plants.

Some extracts of *Ajuga* species from Japan were found to disrupt larvae development of some insects. For example, some methanolic extracts from *Ajuga reptans* var. *reptans* could deterrent the moulting activities on the Mexican Bean Beetle(*Epilachna varivestis* Muls.) An ethanolic extract lowered the ecdysteroid level in cockroach(*Periplaneta americana* L.) larvae. Food treated with acetonitrile extracts of *A. reptans var. reptans* disturbed moulting during the larval development of the fleshfly(*Neobellieria bullata* Parker), and causing early head sclerotization during the wandering phase of the last larvae stadium of *N. bullata* when used with Ohtaki-type wet synchronization. Neo-clerodanes from *Ajuga* species had phagodeterrent activity on the cotton worm(*Spodoptera littoralis* (Boisd.)) as well as on *P. americana*, resulting in a decrease in production and weight of oothecae[1~5].

Our aim here was to test the antifeedent and the insecticide activities of the crude methanolic and acetone extracts of *A. multiflora* Bunge, *A. multiflora* var *serotina* Kitagawa and *A. multiflora* var. *brevispicata* G. Y. Wu et G. Chen to the larvae of *H. cunea* and their natural enemies.

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Materials and Methods

Plants

The plants we used to making the extracts were *A. multiflora, A. multiflora* var *serotina* and *A. multiflora* var. *brevispicata*, which were collected at Anshan and Shenyang in Liaoning Province and Zhalantun in Heilongjiang Province.

Animals

H. cunea and their natural enemies *Trichogramma dendrolimi, Coccinella septempunctata* were gotten from Ansan city of Liaoning Province. The parasitic enemy of eggs *Trichogramma dendrolimi was* reared from the egg of *H. cunea.*

The making of those extracts

Dried plant materials (10 g) were grounded and sonicated with methanol or acetone (2×190 mL) for 5 min. In each case, the extracts combined and solvent removed under vacuum and the residue was redissolved in methanol (10 mL) and stored at -50°C. Six kinds of extracts were made. Those were the *A. multiflora* extracted with methanol (AMM), *A. multiflora* extracted with acetone (AMA), *A. multiflora* var. *serotina* extracted with methanol (AMSM), *A. multiflora* var. *serotina* extracted with acetone (AMSA), *A. multiflora* var. *brevispicata* extracted with methanol (AMBM), *A. multiflora* var. *brevispicata* extracted with acetone (AMBA). Those extracts were diluted 25, 50 or 100 times with water when they were used in the treatment of those animals.

Rearing and treating of those pest and their enemies

H. cunea: The larva of *H. cunea* used were gotten from the stock culture of eggs laid by the adult in the same day. Twenty second instar larvae were reared in each glass pot which was 10 cm in diameter and 15 cm high. The food used to feed those larvae were leaves of *Acer saccharum* Marsh on their twig. Those leaves were soaked in the diluted extracts originated from different species and extracted with different solvents for 3 s, air dried, water cultivated in a small glass tube. Those pots were covered with fine gauze then put into a constant temperature animal rearing room at 25 ℃ and 60%-70% R.H., under light:dark 16:8 h conditions. Ten mL of methanol diluted with water was set up as control group and three repetitions was used. The food for those larvae was changed every three day and at the same time the number of dead larvae was recorded.

T. Dendrolimi The experiment carried out for the testing the effect of those extracts on *T. dendrolimi* were conducted by treating the eggs parasitized by this enemy to see the emergence rate of this trichogrammatids. Those parasitized eggs were gotten by introducing the female adults of *T. dendrolimi* into the cage which containing the eggs just laid by female *H. cunea.* Thirty parasitized eggs were treated by different extracts which were obtained from different plant species and with different concentration for 30 s. Then they were taken out and put in glass pots. The pots and the environments used to rear those eggs were the same as which used to rear those treated larvae of *H. cunea.* The number of adults emerged we recorded each day to see the effects of those extracts on the development of those enemies. Eggs soaked with 10 mL methanol diluted with 25 times water (V/V) were set up as the control group. Three repetitions were used for each treatment.

C. septempunctata To test the influence of those extracts on *C. septempunctata,* the larvae and adults of *C. septempunctata* were collected from the stands of Sugar Maple, *A. saccharum.* Two kinds of treatments were carried out in this experiment. The first, 20 adult or larvae were put into a pot and fed with sufficient larvae of *H. cunea* feeding on the leaves treated with different extracts. The method used to treated those leaves was the same as that used in *H. cunea* experiments. The second method used to treated *C. septempunctata* was to soak the adult or larvae of this species in different extracts for 1 s then rearing them on the untreated larvae of *H. cunea.* The foods for both experiments were changed every two days and at the same time the living status of those *C. septempunctata* was recorded. The rearing condition for those *C. septempunctata* was as the same as that used in the The effects of those extracts on the larvae of *H. cunea* experiments. The untreated group and repetition were also set up.

Results and Discussion

Effects of extracts on larvae of *H. cunea*

In this experiment 6 kinds of extracts were used, those were the *A. multiflora* extracted with methanol (AMM), *A. multiflora* extracted with acetone (AMA), *A. multiflora* var. *serotina* extracted with methanol (AMSM), *A. multiflora* var. *serotina* extracted with acetone (AMSA), *A: multiflora* var. *brevispicata* extracted with methanol (AMBM), *A. multiflora* var. *brevispicata* extracted with acetone (AMBA). All of those extracts (10 mL) were diluted by adding 25, 50 or 100 times of distilled water in it. Ten mL of methanol were also diluted 25 times to treated the leaves used to feed the larvae as the control groups. The leaves used to feed larvae were treated by

those solutions. We have observed the behavior and the number of mortality of those treated pests. The rate of mortality of *H. cunea* was larvae summerized in Table 1.

Table 1. The mortality rate of *H. cunea* larvae treated by some *Ajuga* extracts

kind of extracts	times of added water	number of used larvae	number of dead larvae	rate of mortality (%)	modified mortality rate(%)
AMM	25	60	58	96.7	96.33
	50	60	57	95.0	94.44
	100	60	58	96.7	96.33
AMA	25	60	47	78.3	75.88
	50	60	52	86.7	85.22
	100	60	43	71.7	68.55
AMSM	25	60	57	95.0	94.44
	50	60	56	93.3	92.56
	100	60	56	93.3	92.56
AMSA	25	60	48	80.0	77.78
	50	60	50	83.3	81.44
	100	60	47	78.3	75.89
AMBM	25	60	58	96.7	96.33
	50	60	56	93.3	92.56
	100	60	54	90.0	88.89
AMBA	25	60	51	85.0	83.33
	50	60	50	83.3	81.44
	100	60	43	71.7	68.55
Control		180	18	10.0	

The results in Table 1 showed that all those extracts could lead the death of the second instar larvae of *H. cunea*. The modified rate of mortality of this pest is from 68.55% to 96.33%. The average death rate caused by those extracts was 85.70%. The mortality rate caused by the extracts drawn with methanol was from 88.89% to 96.33%, which was significantly higher than that caused by acetone extracts. It seems that there was no significant difference between the mortality rate caused by different concentration. Even the extracts drawn with methanol and diluted 100 times was very active in the leading of those larvae to death. The average death rate was up to 92.59%. All of those data proved that those *Ajuga* extracts had a very high larva killing activity to *H. cunea*. This *Ajuga* plant could be used as a kind of insecticide in the integrated control of *H. cunea*.

Effect of extracts on *T. dendrolimi*

In these experiments 3 kinds of extracts were used, those were the *A. multiflora* extracted with methanol (AMM), *A. multiflora* var. *serotina* extracted with methanol (AMSM), *A. multiflora* var. *brevispicata* extracted with methanol (AMBM). Those extracts were diluted into two concentrations by adding 25 times or 50 times (V/V) distilled water. The emergence rate of those trichogrammatids in treated eggs was observed every day to evaluated the effects of those extracts on the development of this kind of parasitic enemy. The results of this experiment were calculated and listed in Table 2.

Table 2. The effect of some *Ajuga* extracts on the emergence rate of *T. dendrolimi*

kinds of extracts	times of dilution	Number of repetition	Number of eggs treated	Number of *T. dendrolimi*	Number of emergence	rate of emergence (%)	average rate of emergence(%)
AMM	25	1	30	467	332	71.09	75.21
		2	30	497	379	76.26	
		3	30	516	404	78.29	
	50	1	30	324	221	68.21	72.54
		2	30	576	448	79.01	
		3	30	618	435	70.39	
AMBM	25	1	30	567	421	74.25	73.88
		2	30	412	317	76.94	
		3	30	457	322	70.46	
	50	1	30	534	342	64.04	69.95
		2	30	416	292	70.19	
		3	30	369	279	75.61	
AMSM	25	1	30	296	197	66.55	72.38
		2	30	342	267	78.07	
		3	30	564	409	72.52	
	50	1	30	467	339	71.22	69.09
		2	30	521	344	66.03	
		3	30	397	278	70.03	
Control			90	1 356	1 004	74.04	74.04

From the data listed in Table 2, we could found that there was no significant difference between the average

rate of emergence of the extracts treated groups and the control group. The average emergence rate of the control was 74.04%. The average emergence rates of those extracts treated groups were from 69.09% to 75.21%. Some of the emergence rate in the treated groups even higher than that of the control group.

The observation of the behavior of those emerged adults also found that all the adults were the same. There was no difference between the length of the body and the color of the body between the adults of the treated groups and the control one.

Until now, we did not found any evidence shown that those extracts had any influence the emergence and the development of *T. dendrolimi.*

Effects of extracts on *C. septempunctata* experiments

In this experiment 2 kinds of extracts were used, those were the *A. multiflora* extracted with methanol (AMM), *A. multiflora* var. *brevispicata* extracted with methanol (AMBM). All of those extracts (10 mL) were diluted by adding 25, distilled water in it. Ten ml of methanol were also diluted 25 times as the control groups. The larvae and adults were either soaked with those extracts or fed with the *H. cunea* larvae feeding on the treated *A. saccharum* leaves. The behavior and the number of mortality of *C. septempunctata* were observed here. The rates of mortality of larvae and adults were summerized in Table 3 and 4.

Table 3. The mortality rate of *C. septempunctata* larvae treated by some of the *Ajuga* extracts

kinds of extracts	times of dilution	No. of repetition	No. of animals	No. of mortality	rate of mortality (%)	average rate of mortality (%)
			Soaking the body			
AMM	25	1	20	3	15	20.00
	25	2	20	2	10	
	25	3	20	7	35	
AMBM	25	1	20	2	10	18.33
	25	2	20	4	20	
	25	3	20	5	25	
Control	25		60	10	16.67	16.67
			Feeding on the treated larvae			
AMM	25	1	20	7	35	16.67
	25	2	20	2	10	
	25	3	20	1	5	
AMBM	25	1	20	4	20	18.33
	25	2	20	3	15	
	25	3	20	3	15	
Control	25		60	11	18.33	18.33

The results listed in Table 3 and 4 showed that, the mortality rate of third instar larva and adult which soaked in the extracts of AMM and AMBM were 20%, 18.33% , 5% and 0% respectively. The mortality rate of larvae and adults treated with the control group were 16.68% and 6.7%. The mortality of the larvae and adults feeding on the larvae of *H. cunea* living on the AMM and AMBM treated leaves were 16.67%, 18.33%, 5% and 3.3% respectively. The mortality rate of larvae and adults treated with the control group were 18.33% and 3.3%. There was no significant difference between the extracts treated group and the control group in the mortality rate aspect. We could conclude that those methanolic extracts gotten from *A. multiflora* and *A. multiflora* var. *brevispicata* had no significant effects on the mortality of the larvae and adults of *C. septempunctata.* Those extracts could be used in the control of *H. cunea* safely.

Table 4. The mortality rate of *C. septempunctata* adults treated by some of the *Ajuga* extracts

kinds of extracts	times of dilution	No. of repetition	No. of animals	No. of mortality	rate of mortality (%)	average rate of mortality (%)
			Soaking the body			
AMM	25	1	20	2	10	5.0
	25	2	20	1	5	
	25	3	20	0	0	
AMBM	25	1	20	0	0	0.0
	25	2	20	0	0	
	25	3	20	0	0	
Control	25		60	4	6.7	6.7
			Feeding on the treated larvae			
AMM	25	1	20	1	5	5.0
	25	2	20	2	10	
	25	3	20	0	0	
AMBM	25	1	20	1	5	3.3
	25	2	20	1	5	
	25	3	20	0	0	
Control	25		60	2	3.3	3.3

Conclusion

All those extracts gotten from *A. multiflora* and *A. multiflora* var. *brevispicata and A. multiflora var. serotina* were very effective to led the death of the second instar larvae of *H. cunea.* The modified rate of mortality of this pest is from 68.55% to 96.33%. The average death rate caused by those extracts was 85.70%. The mortality rate caused by the extracts drawn with methanol was from 88.89% to 96.33%, which was significantly higher than that caused by acetone extracts. It seems that there was no significant difference between the mortality rate caused by different concentration. Even the extracts

drawn with methanol and diluted 100 times was very active in the leading of those larvae to death. The reason for this was the chemicals contained in those extracts which was active in the leading of the death was molting pheromone, so it could show very high activity even in a low concentration level. The average death rate was up to 92.59%.

There was no significant difference between the average rate of emergence of the extracts treated *T. dendrolimi* and the control group. The average emergence rate of the control was 74.04%. The average emergence rates of those extracts treated groups were from 69.09% to 75.21%. There was no difference between the length of the body and the color of the body between the adults of the treated groups and the control one. We did not found any evidence shown that those extracts had any influence the emergence and the development of *T. dendrolimi*.

The mortality rate of third instar larva and adult of *C. septempunctata* which either soaked in the extracts of AMM and AMBM or feeding on the larvae of *H. cunea* living on the AMM and AMBM treated leaves had no significant difference with their control group's. We could conclude that those methanolic extracts gotten from *A. multiflora* and *A. multiflora* var. *brevispicata* had no significant effects on the mortality of the larvae and adults of *C. septempunctata*. Those extracts could be used in the control of *H. cunea* safely.

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